

CLAIMS

What is claimed is:

1. A method for delivering NO to cells in a mammal,
comprising administering low molecular weight
5 nitrosating agent to the mammal.
2. A method for increasing O₂-delivery capacity of
hemoglobin in a mammal, comprising administering a low
molecular weight NO donating agent to the mammal.
3. A method for scavenging oxygen free radicals in a
10 mammal, comprising administering low molecular weight
nitrosating agent to the mammal.
4. A method for preserving a living organ ex vivo,
comprising perfusing the organ with a composition
comprising nitrosated hemoglobin and low molecular
15 weight thiol or NO donating agent.
5. A method for treating a blood borne disease in a
patient, comprising the steps of:
- 20 a) isolating the patient's red blood cells;
b) treating the patient's red blood cells with S-
nitrosothiol; and
c) readministering to the patient the red blood
cells.
6. The method of Claim 5, wherein the blood borne disease
is malaria.
- 25 7. A method for treating a disease or medical disorder in
a mammal, comprising administering to the mammal a
nitrosating agent.

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8. The method of Claim 7 in which the nitrosating agent is selected for rapid entry into the target cell.

9. The method of Claim 7, wherein the disease or medical disorder is selected from the group consisting of:

5 shock, angina, stroke, reperfusion injury, acute lung injury, sickle cell anemia and infection of red blood cells.

10. A composition comprising SNO-Hb[FeII]O₂ which is S-nitrosylated without detectable oxidation of the heme Fe.

11. A method for making SNO-Hb[FeII]O₂, which is specifically S-nitrosylated on thiol groups, comprising incubating excess nitrosating agent with purified hemoglobin in the presence of oxygen.

12. The method of Claim 11 in which the nitrosating agent is a low molecular weight S-nitrosothiol.

13. A composition comprising SNO-Hb[FeII] which is S-nitrosylated without detectable oxidation of the heme Fe.

14. A method for making SNO-Hb[FeII], which is specifically S-nitrosylated on thiol groups, comprising incubating excess nitrosating agent with purified hemoglobin in the absence of oxygen.

15. The method of Claim 14 in which the nitrosating agent is a low molecular weight S-nitrosothiol.

16. A method for regulating delivery of oxygen and NO, in various redox forms, in a mammal, comprising

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~~administering to the mammal a mixture of a low molecular weight thiol or nitrosothiol and hemoglobin or nitrosated hemoglobin, selected for the oxidation state of the heme iron and for the oxygenation state.~~

- 5 17. A method for delivering NO in a mammal, comprising administering to the mammal a blood substitute comprising nitrosated hemoglobin.

18. The method of Claim 17, in which the blood substitute comprises nitrosated hemoglobin and low molecular weight S-nitrosothiol.
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19. A method for scavenging oxygen free radicals and NO \cdot in a mammal, comprising administering to the mammal a blood substitute comprising nitrosated hemoglobin.

- 15 20. A method for reducing blood pressure in a mammal, comprising administering nitrosated hemoglobin to the mammal.

21. A method for treating a disease in a mammal, comprising administering a form of nitrosated or nitrated hemoglobin to the mammal, wherein the disease is selected from the group consisting of heart disease, brain disease, vascular disease, atherosclerosis, lung disease and inflammation.
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22. A method for treating a medical condition in a mammal, comprising administering a form of nitrosated hemoglobin to the mammal, wherein the medical condition is selected from the group consisting of stroke, angina and acute respiratory distress.
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23. A method for enhancing the preservation of an excised organ, comprising storing the organ in a solution comprising SNO-Hb(FeII)O₂.

5 24. A method for treating a human with sickle cell anemia comprising administering to the human a preparation comprising SNO-Hb(FeII)O₂.

25. The method of Claim 24 in which the preparation comprises SNO-Hb(FeII)O₂ and a thiol.

10 26. The method of Claim 24 in which the preparation comprises SNO-Hb(FeII)O₂ and an S-nitrosothiol.

15 27. A method for treating a patient having a disease or medical condition characterized by abnormalities of nitric oxide and oxygen metabolism, comprising administering to the patient an effective amount of a preparation comprising nitrosated hemoglobin.

28. The method of Claim 27 in which the disease or medical condition is selected from the group consisting of: heart disease, lung disease, sickle-cell anemia, stroke, sepsis or organ transplantation.

20 29. A blood substitute comprising nitrosated or nitrated hemoglobin.

25 30. A method for treating a disorder resulting from platelet activation or adherence in an animal or human, comprising administering a composition comprising nitrosated or nitrated hemoglobin in a therapeutically effective amount.

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31. The method of Claim 30 wherein the disorder is selected from the group consisting of: myocardial infarction, pulmonary thromboembolism, cerebral thromboembolism, thrombophlebitis, sepsis and unstable angina.

5 32. A method for preventing thrombus formation in an animal or human, comprising administering a composition comprising nitrosated hemoglobin in a therapeutically effective amount.

10 33. A method for regulating platelet activation in an animal or human, comprising administering, in a therapeutically effective amount, a composition comprising a substance which controls the allosteric equilibrium or spin state of hemoglobin.

15 34. The method of Claim 33 in which the substance converts the allosteric state of hemoglobin from R-structure to T-structure.

20 35. A method for forming polynitrosated hemoglobin, comprising combining hemoglobin with an excess of S-nitrosothiol over hemoglobin tetramer in an aqueous solution, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on hemoglobin.

25 36. A method for forming polynitrosated or polynitrated hemoglobin in which heme Fe is in the FeII state, comprising combining hemoglobin with an NO donating compound, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby forming polynitrosated or polynitrated hemoglobin, and reacting the polynitrosated or

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polynitrated hemoglobin with a reagent which selectively reduces FeIII to FeII.

37. The method of Claim 36 in which the reagent which selectively reduces FeIII to FeII is a cyanoborhydride.

5 38. The method of Claim 36 in which the reagent which selectively reduces FeIII to FeII is methemoglobin reductase.

39. A composition comprising polynitrosated hemoglobin.

10 40. Method for treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder, in an animal or human, comprising administering to the animal or human nitrosyl-heme-containing donors of NO.

15 41. The method of Claim 40 wherein the nitrosyl-heme-containing donor of NO is nitrosylhemoglobin.

20 42. Method for making stable nitrosyl-deoxyhemoglobin comprising adding NO to deoxyhemoglobin in an aqueous solution such that the ratio of NO:heme is less than about 1:100 or greater than about 0.75.

43. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin and buffer having a pK of at least about 9.4, at a concentration of approximately 10 mM to 200 mM, at pH 7.4.

25 44. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin in approximately 10 mM phosphate buffer at pH 7.4.

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45. A composition comprising nitrosyl-deoxyhemoglobin in a physiologically compatible buffer, wherein the ratio of NO:heme is less than about 1:100 or greater than about 0.75.

5 46. Method for making nitrosyl-oxyhemoglobin comprising adding NO to oxyhemoglobin in an aqueous solution such that the ratio of NO:hemoglobin is less than about 1:30.

47. Hemoglobin conjugated to an NO-donor.

10 48. Hemoglobin of Claim 47, wherein the NO-donor is selected from the group consisting of: diazeniumdiolates, nitroprusside, nitroglycerin and nitrosothiol.

15 49. A composition comprising hemoglobin and one or more NO-donors.

20 50. A method for treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder, in an animal or human, comprising administering a heme-based blood substitute and inhaled NO to the animal or human.

51. A method for delivering CO to the tissues in an animal or human, comprising administering CO-derivatized hemoglobin to the animal or human.

25 52. A method of treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder in an animal or human,

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comprising administering both CO-derivatized hemoglobin and a nitrosated hemoglobin to the animal or human.

53. Nitrosylhemoglobin conjugated to one or more electron acceptors.

5 54. Nitrosylhemoglobin of Claim 53, wherein the electron acceptor is selected from the group consisting of: superoxide dismutase, stable nitroxide radicals, and oxidized forms of nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, flavin
10 adenine dinucleotide, flavin mononucleotide, ascorbate and dehydroascorbate.

55. A composition comprising nitrosylhemoglobin and one or more electron acceptors.

56. Hemoglobin conjugated to nitric oxide synthase.

15 57. Hemoglobin of Claim 56, wherein the nitric oxide synthase is nitric oxide synthase of neurons.

58. A composition comprising hemoglobin and nitric oxide synthase.

59. A method for making erythrocytes comprising
20 nitrosylhemoglobin, comprising incubating deoxygenated erythrocytes in a solution comprising NO.

60. Erythrocytes comprising nitrosylhemoglobin.

25 61. A method for treating shock in an animal or human comprising administering hemoglobin α -chains to the animal or human.

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62. A method for ~~treating or preventing a disease or~~
medical disorder which can be ameliorated by delivery
of NO or its biological equivalent to tissues affected
by the disease or medical disorder, ~~in an animal or~~
5 human, comprising administering hemoglobin β -chains to
the animal or human.

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